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(54) Title: TRIPLIDE 5,6-DERIVATIVES AS IMMUNOMODULATORS AND ANTICANCER AGENTS

(57) Abstract: Compounds useful as immunosuppressive, anti-inflammatory and anticancer agents and methods of their preparation and use are described. The compounds are analogs or derivatives of triplide and related compounds, modified at the 5- and/or 6-position relative to the naturally occurring compounds.



WO 2005/062913 A2

Triptolide 5,6-Derivatives as Immunomodulators and Anticancer Agents**Field of the Invention**

The present invention relates to compounds useful as immunosuppressive,
5 anti-inflammatory and anticancer agents.

References

- Dai, D. *et al.*, U.S. Patent No. 6,569,893 (2003).
Fidler, J.M. *et al.*, U.S. Patent No. 6,620,843 (2003).
10 Gleichmann, E. *et al.*, *Immunol. Today* 5:324 (1984).
Jung, M.J. *et al.*, US 5,972,998 (1999).
Jung, M.J. *et al.*, US 6,004,999 (1999).
He, Q. *et al.*, *Beijing Da Xue Xue Bao* 35:252-5 (Jun 2003).
Krishna, G. *et al.*, *Am. J. of Pathology* 158(3):997-1004 (Mar 2001).
15 Kupchan, S.M. *et al.*, *J. Am. Chem. Soc.* 94:7194 (1972).
Kupchan, S.M. *et al.*, U.S. Patent No. 4,005,108 (1977).
Lipsky *et al.*, U.S. Patent No. 5,294,443 (1994).
Ma *et al.*, *J. Chin. Pharm. Sci.* 1:12 (1992).
Murase, N. *et al.*, *Transplantation* 55:701 (1993).
20 Ning, L. *et al.*, *Pure and Applied Chemistry* 75(2-3):389-392 (2003).
Ning, L. *et al.*, *Tetrahedron* 59(23):4209-4213 (2003).
Ono and Lindsey, *J. Thor. Cardiovasc. Surg.* 57(2):225-29 (1969).
Panchagnula, R. and Thomas, N.S., *Intl J Pharmaceutics* 201(2):131-150 (2000).
Pu, L. *et al.*, *Zhongguo Yaoli Xuebao* 11:76 (1990).
25 Qi, Y. *et al.*, U.S. Patent 5,663,335 (1997).
Qi, Y. *et al.*, U.S. Patent 5,962,516 (1999).
Qian, S. *et al.*, U.S. Patent No. US 5,430,054 (1995).
Strobel, G.A. *et al.*, U.S. Patent No. 5,648,376 (1997).
Wang, J. and Morris, R.E., *Transplantation Proc.* 23:699 (1991).
30 Wang, X. *et al.*, PCT Pubn. No. WO 2002/17931 (2002).
Wiedmann, T. *et al.*, U.S. Patent No. 5,843,452 (1998).
Zhou, Y.X. *et al.*, *Ai Zheng* 21(10):1108-8 (Oct 2002).

Background of the Invention

Immunosuppressive agents are used in the treatment of autoimmune disease and in treating or preventing transplantation rejection, including graft-versus-host disease (GVHD). Common immunosuppressive agents include azathioprine, corticosteroids, cyclophosphamide, methotrexate, 6-mercaptopurine, vincristine, and cyclosporin A. In general, none of these drugs are completely effective, and most are limited by severe toxicity. For example, cyclosporin A, a widely used agent, is significantly toxic to the kidney. In addition, doses needed for effective treatment may increase the patient's susceptibility to infection by a variety of opportunistic invaders.

10 A number of compounds derived from the Chinese medicinal plant *Tripterygium wilfordii* (TW) have been identified as having immunosuppressive activity, e.g. in the treatment of autoimmune disease, and in treating or preventing transplantation rejection, including the treatment of graft-versus-host disease (GVHD), a condition in which transplanted marrow cells attack the recipient's cells. See, for example, U.S. Patent Nos. 15 4,005,108, 5,294,443, 5,843,452, and 5,648,376, cited above.

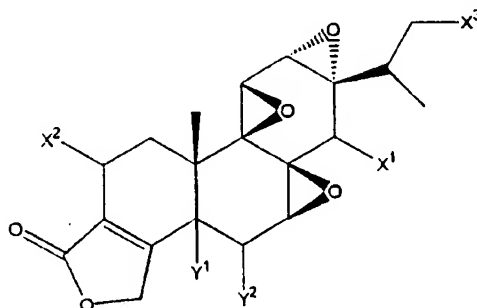
Triptolide and certain of its derivatives have also been reported to show anticancer activity. See, for example, Kupchan *et al.*, 1972, 1977, as well as co-owned U.S. Patent No. 6,620,843, which is hereby incorporated by reference.

Triptolide derivatives often provide other benefits relative to native triptolide in areas such as formulation, pharmacokinetics and biodistribution, by virtue of differences in solubility and/or their activity as prodrugs. Such derivatives are described, for example, in Jung *et al.*, U.S. Patent Nos. 5,972,998 and 6,004,999, and Qian *et al.*, U.S. Patent No. 5,430,054, as well as co-owned U.S. Patent Nos. 6,150,539 (Triptolide prodrugs having high aqueous solubility), 5,962,516 (Immunosuppressive compounds and methods), 25 5,663,335 (Immunosuppressive compounds and methods), and 6,569,893 (Amino acid derivatives of triptolide compounds as immune modulators and anticancer agents).

In general, these disclosures are directed to derivatives and/or prodrugs produced by synthetic modification of triptolides at the epoxy rings, hydroxyl groups, or the lactone ring. None of these earlier disclosures describe modifications at the 5 or 6 position of 30 triptolide, as disclosed herein.

Summary of the Invention

In one aspect, the invention provides compounds which are useful for immunosuppressive, anti-inflammatory and anticancer therapy. The compounds are represented, in one embodiment, by Formula I:



I

where

X^1 is OR^1 , where R^1 is selected from hydrogen, $C(=O)R^2$, and $C(=O)OR^2$, where R^2 is selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl;

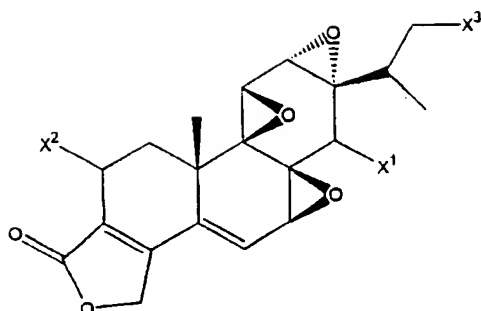
X^2 and X^3 are independently OR^1 or hydrogen, at least one of X^2 and X^3 being hydrogen; and where

(i) Y^1 is hydrogen and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl, ethenyl ($-CH=CH_2$), $-CH_2COOR^4$, $N(R^4)_2$, and SR^4 , where each R^4 is independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, or, in the case of $N(R^4)_2$, taken together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, wherein the ring atoms include at most 3 heteroatoms; or

(ii) Y^1 is hydroxyl and Y^2 is selected from the group consisting of hydrogen, hydroxyl, halogen, cyano, $N(R^4)_2$, and SR^4 ; or

(iii) Y^1 and Y^2 taken together form an epoxide ring.

The compounds are represented, in another embodiment, by Formula II:



II

where X^1 , X^2 and X^3 are as defined above.

Preferably, X^1 is selected from OH and $OC(=O)R^2$ (that is, R^1 is selected from
 5 hydrogen and $C(=O)R^2$). When R^1 is $C(=O)R^2$, R^2 is preferably selected from alkyl, aryl,
 aralkyl, and alkoxyalkyl, and is more preferably selected from alkyl, *e.g.* methyl or ethyl;
 aryl, *e.g.* phenyl, and aralkyl, *e.g.* benzyl. In one embodiment, R^2 is methyl.

When either of X^2 and X^3 is OR^1 , preferred embodiments of R^1 are as described
 above.

10 In one embodiment, each of X^2 and X^3 is hydrogen.

One embodiment of the compounds of formula I is that in which (i) Y^1 is hydrogen
 and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl,
 ethenyl, $-CH_2COOR^4$, $N(R^4)_2$, and SR^4 . Preferred embodiments of this class include those
 in which Y^2 is hydroxyl, fluoro, chloro, bromo, cyano, $-CH_2COOR^4$, or $N(R^4)_2$. In further
 15 preferred embodiments, Y^2 is hydroxyl, fluoro, chloro, bromo, or cyano. In one preferred
 embodiment, Y^2 is hydroxyl; in another preferred embodiment, Y^2 is cyano.

A further embodiment of the compounds of formula I is that in which (ii) Y^1 is
 hydroxyl and Y^2 is selected from the group consisting of hydrogen, hydroxyl, halogen,
 cyano, $N(R^4)_2$, and SR^4 . Preferred embodiments of this class include those in which Y^2 is
 20 hydrogen, hydroxyl, fluoro, chloro, bromo, or cyano. In one embodiment, Y^2 is
 hydrogen; in a further embodiment, Y^2 is hydroxyl.

A further embodiment of the compounds of formula I is that in which (iii) Y^1 and Y^2
 taken together form an epoxide ring.

The groups defined above as R^2 , R^3 , and R^4 , when selected from alkyl, alkenyl, and
 25 alkynyl, preferably have up to six carbon atoms, more preferably up to four carbons, and,
 in selected embodiments, one or two carbons. When selected from cycloalkyl or
 cycloalkenyl, they preferably have 3 to 7, or, for cycloalkenyl, 5 to 7 carbon atoms. When

selected from aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, the alkyl components of these groups preferably have up to six carbon atoms, more preferably up to four carbons, and, in selected embodiments, one or two carbons. In further selected embodiments, each of these groups is independently selected from alkyl, aryl, aralkyl, and
5 alkoxyalkyl.

In other aspects, the invention provides a method of effecting immunosuppression, and a method of inducing apoptosis in a cell. The methods comprise administering to a subject in need of such treatment, or contacting said cell, respectively, with an effective amount of a compound of formula I or II as described above. The compound is typically
10 provided in a pharmaceutically acceptable carrier. Specific embodiments of the methods may employ any of the specific embodiments of formula I or II described herein.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

15

Brief Description of the Drawings

Fig. 1 shows dose-dependent induction of apoptosis in Jurkat cells by a compound of the invention, 5- α -hydroxy triptolide (designated PG701), in comparison with triptolide (designated PG490) (Example 8);

20 Fig. 2 shows the cytotoxic effect in Jurkat cells of a compound of the invention, 14-acetyl-5,6-didehydro triptolide (designated PG746), in comparison with triptolide (designated PG490) (Example 9); and

Figs. 3 and 4 show inhibition of IL-2 production in Jurkat cells by invention compounds PG701 and PG746, respectively, in comparison with triptolide (PG490)
25 (Example 10).

Detailed Description of the Invention

I. Definitions

The terms below have the following meanings unless indicated otherwise.

30 "Alkyl" refers to a fully saturated acyclic moiety consisting of carbon and hydrogen, which may be linear or branched. Examples of alkyl groups are methyl, ethyl, n-butyl, t-butyl, n-heptyl, and isopropyl. Generally preferred are lower alkyl groups, having one to

six carbon atoms, as exemplified by methyl, ethyl, n-butyl, i-butyl, t-butyl, isoamyl, n-pentyl, and isopentyl. In other embodiments, lower alkyl includes groups having one to four carbon atoms, or 1-2 carbon atoms (methyl and ethyl).

"Cycloalkyl" refers to a fully saturated cyclic moiety consisting of carbon and hydrogen, having three to eight carbon atoms, preferably three to six carbons atoms; *e.g.* cyclopropyl or methylcyclopentyl. "Cycloalkenyl" refers to an unsaturated cyclic moiety consisting of carbon and hydrogen, having five to eight carbon atoms, preferably five or six carbon atoms.

"Alkenyl" refers to an unsaturated acyclic moiety consisting of carbon and hydrogen, which may be linear or branched, having one or more double bonds. Generally preferred are lower alkenyl groups, having two to six, or two to four, carbon atoms. "Alkynyl" refers to an unsaturated acyclic moiety consisting of carbon and hydrogen, which may be linear or branched, containing one or more triple bonds. Generally preferred are lower alkynyl groups, having two to six, or two to four, carbon atoms.

"Aryl" refers to a substituted or unsubstituted monovalent aromatic radical, generally having a single ring (*e.g.*, benzene) or two condensed rings (*e.g.*, naphthyl), where monocyclic aryl groups are preferred. The term includes heteroaryl groups, which are aromatic ring groups having one or more nitrogen, oxygen, or sulfur atoms in the ring, such as furyl, pyrrole, pyridyl, and indole. By "substituted" is meant that one or more ring hydrogens in the aryl group, preferably one or two ring hydrogens, is replaced with a group preferably selected from fluorine, chlorine, bromine, methyl, ethyl, hydroxy, hydroxymethyl, nitro, amino, methylamino, dimethylamino, methoxy, halomethoxy, and halomethyl.

"Acyloxyalkyl" refers to a substituent of the form -R-O-(C=O)-R', where R is alkyl, preferably having up to six carbon atoms, and R' is selected from alkyl, alkenyl, alkynyl, aryl, and aralkyl, where R' preferably comprises lower alkyl, lower alkenyl, or lower alkynyl (*i.e.* C₂-C₆) groups and monocyclic aryl groups.

"Aralkyl" refers to an alkyl, preferably lower (C₁-C₄, more preferably C₁-C₂) alkyl, substituent which is further substituted with an aryl group, preferably a monocyclic aryl group; examples are benzyl and phenethyl. Also included is fluorenylmethyl, a component of the widely employed Fmoc (fluorenylmethoxycarbonyl) protecting group.

A "5- to 7-member heterocyclic ring whose ring atoms are selected from the group

consisting of carbon, nitrogen, oxygen and sulfur, wherein the ring atoms include at most 3 heteroatoms" preferably refers to a non-aromatic ring, and includes such nitrogen heterocycles as pyrrolidine, piperidine, and morpholine.

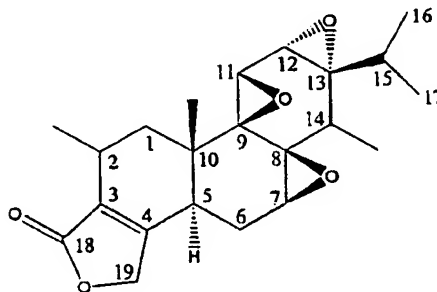
The term "pharmaceutically acceptable salt" encompasses carboxylate salts having organic and inorganic cations, such as alkali and alkaline earth metal cations (for example, lithium, sodium, potassium, magnesium, barium and calcium); ammonium; or organic cations, for example, dibenzylammonium, benzylammonium, 2-hydroxyethyl ammonium, bis(2-hydroxyethyl) ammonium, phenylethylbenzylammonium, dibenzylethylene diammonium, and the like. Other cations encompassed by the above term include the protonated form of procaine, quinine and N-methylglucosamine, and the protonated forms of basic amino acids such as glycine, ornithine, histidine, phenylglycine, lysine, and arginine.

The term also includes salts formed by standard acid-base reactions with basic groups, such as amino groups, having a counterion derived from an organic or inorganic acid. Such counterions include chloride, sulfate, phosphate, acetate, succinate, citrate, lactate, maleate, fumarate, palmitate, cholate, glutamate, glutarate, tartrate, stearate, salicylate, methanesulfonate, benzenesulfonate, sorbate, picrate, benzoate, cinnamate, and the like.

II. Triptolide 5,6-Derivatives

A. Compounds and Preferred Embodiments

For the purposes of the current disclosure, the following numbering scheme is used for triptolide and triptolide derivatives:

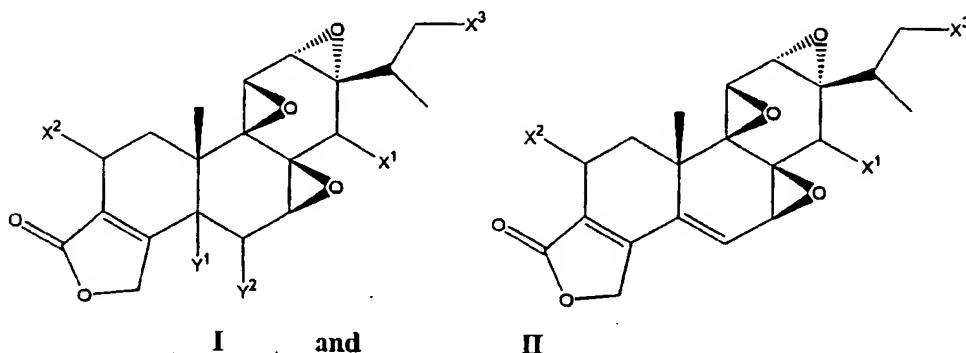


The compounds of the invention are derivatives of triptolide resulting from modification at the 5 and/or 6 position.

In one embodiment, the compound is 5 α -hydroxytriptolide, which may be prepared as described below. This compound may be used as an entry point for preparation of other compounds modified at the 5 and/or 6 position.

In addition, the hydroxyl groups of the starting material, *e.g.* triptolide, triptidiolide
5 (2-hydroxytriptolide) or 16-hydroxytriptolide, may be derivatized as esters or carbonates.

The compounds are represented by Formulas I and II below:



10 where

X^1 is OR^1 , where R^1 is selected from hydrogen, $C(=O)R^2$, and $C(=O)OR^2$, where R^2 is selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl;

X^2 and X^3 are independently OR^1 or hydrogen, at least one of X^2 and X^3 being
15 hydrogen.

In formula I, Y^1 and Y^2 are defined according to one of the following three embodiments:

(i) Y^1 is hydrogen and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl, ethenyl, $-CH_2COOR^4$, $N(R^4)_2$, and SR^4 . Preferred embodiments of
20 this class include those in which Y^2 is hydroxyl, fluoro, chloro, bromo, cyano, $-CH_2COOR^4$, or $N(R^4)_2$. In further preferred embodiments, Y^2 is hydroxyl, fluoro, chloro, bromo, or cyano. In one preferred embodiment, Y^2 is hydroxyl; in another preferred embodiment, Y^2 is cyano.

(ii) Y^1 is hydroxyl and Y^2 is selected from the group consisting of hydrogen,
25 hydroxyl, halogen, cyano, $N(R^4)_2$, and SR^4 . Preferred embodiments of this class include those in which Y^2 is hydrogen, hydroxyl, fluoro, chloro, bromo, or cyano. In one embodiment, Y^2 is hydrogen; in a further embodiment, Y^2 is hydroxyl.

(iii) Y^1 and Y^2 taken together form an epoxide ring.

Each group R^4 is independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, or, in the case of $N(R^4)_2$, taken together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, where the ring atoms include at most 3 heteroatoms.

Preferably, X^1 is selected from OH and $OC(=O)R^2$ (that is, R^1 is selected from hydrogen and $C(=O)R^2$). When R^1 is $C(=O)R^2$, R^2 is preferably selected from alkyl, aryl, aralkyl, and alkoxyalkyl, and is more preferably selected from alkyl, e.g. methyl or ethyl; aryl, e.g. phenyl, and aralkyl, e.g. benzyl. In one embodiment, R^2 is methyl.

When either of X^2 and X^3 is OR^1 , preferred embodiments of R^1 are as described above.

In one embodiment, each of X^2 and X^3 is hydrogen.

The groups defined above as R^2 , R^3 , and R^4 , when selected from alkyl, alkenyl, and alkynyl, preferably have up to six carbon atoms, more preferably up to four carbons, and, in selected embodiments, one or two carbons. When selected from cycloalkyl or cycloalkenyl, they preferably have 3 to 7, or, for cycloalkenyl, 5 to 7 carbon atoms. When selected from aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, the alkyl components of these groups preferably have up to six carbon atoms, more preferably up to four carbons, and, in selected embodiments, one or two carbons. In further selected embodiments, each of these groups is independently selected from alkyl, aryl, aralkyl, and alkoxyalkyl.

B. Preparation of Invention Compounds

The compounds of the invention may be prepared from triptolide, triptidiolide (2-hydroxy triptolide) or 16-hydroxytriptolide. Triptolide can be obtained from the root xylem of the Chinese medicinal plant *Tripterygium wilfordii* (TW) or from other known sources. The TW plant is found in the Fujian Province and other southern provinces of China; TW plant material can generally be obtained in China or through commercial sources in the United States. Methods for preparing triptolide, triptidiolide and 16-hydroxytriptolide are known in the art and are described, for example, in Kupchan *et al.* (1972, 1977); Lipsky *et al.* (1994); Pu *et al.* (1990); and Ma *et al.* (1992).

Compounds of formula I above in which $Y^1 = OH$ and $Y^2 = H$, e.g. 5 α -hydroxy triptolide (designated herein as PG701), may be prepared by reaction of the starting triptolide compound with selenium dioxide, as illustrated in Example 1 below. Such compounds may then be used for preparation of compounds of formula II by dehydration.

- 5 This is illustrated in Examples 2-3 below, where the free 14-hydroxyl group is first derivatized as acetyl, and (diethylamino)sulfur trifluoride (DAST) is used for dehydration.

The 5,6-olefin of formula II can then be used to prepare further compounds of formula I in which

- (i) Y^1 is hydrogen and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl, ethenyl, $-CH_2COOR^4$, $N(R^4)_2$, and SR^4 , where each R^4 is independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, or, in the case of $N(R^4)_2$, taken together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, wherein the
- 10 ring atoms include at most 3 heteroatoms; or

(ii) Y^1 is hydroxyl and Y^2 is selected from the group consisting of hydrogen, hydroxyl, halogen, cyano, $N(R^4)_2$, and SR^4 ; or

(iii) Y^1 and Y^2 taken together form an epoxide ring.

- For example, compounds of formula I in which Y^1 is hydrogen and Y^2 is hydroxyl can be prepared by hydration of the 5,6-olefin of formula II, according to known procedures, e.g. as illustrated in Example 4 below. Compounds of formula I in which Y^1 is hydrogen and Y^2 is a halogen can be prepared by reaction of the hydroxylated compound with a halogenation reagent, e.g. CBr_4 for production of the bromide. Alternatively, the 5,6-olefin may be reacted with a hydrohalic acid HX or its synthetic
- 20 equivalent, in accordance with known procedures.

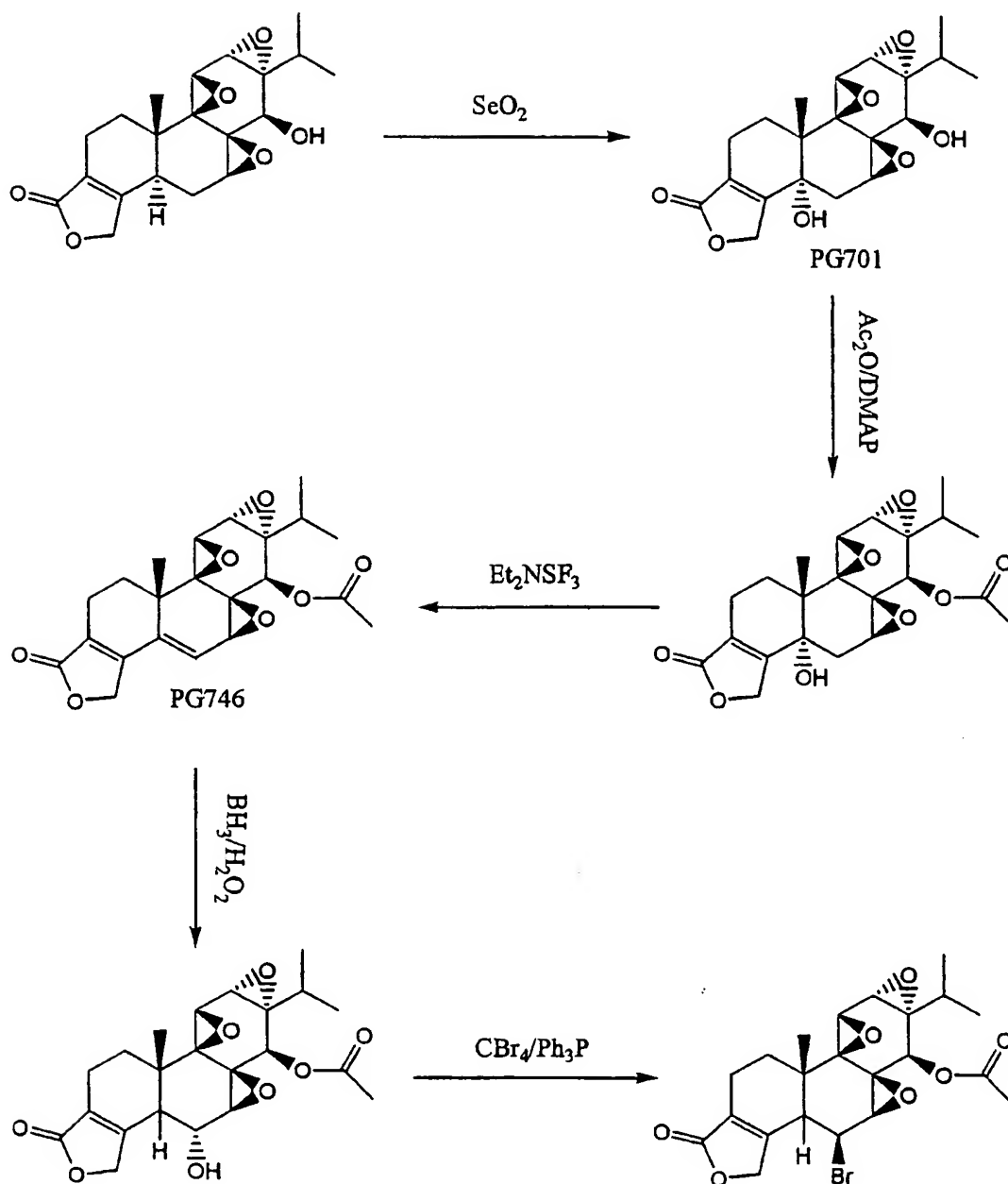
Compounds of formula I in which Y^1 is hydrogen and Y^2 is selected from the group consisting of cyano, nitromethyl, ethenyl, $-CH_2COOR^4$, $N(R^4)_2$, and SR^4 can be formed by Michael addition of a nucleophilic species to the conjugated olefin of formula II, as illustrated in Example 7 for addition of a cyano group.

- 30 Compounds of formula I in which Y^1 and Y^2 taken together form an epoxide ring can be prepared by epoxidation of the 5,6-olefin of formula II, according to known procedures, e.g. using MCPBA, as illustrated in Example 5 below. Ring-opening of the

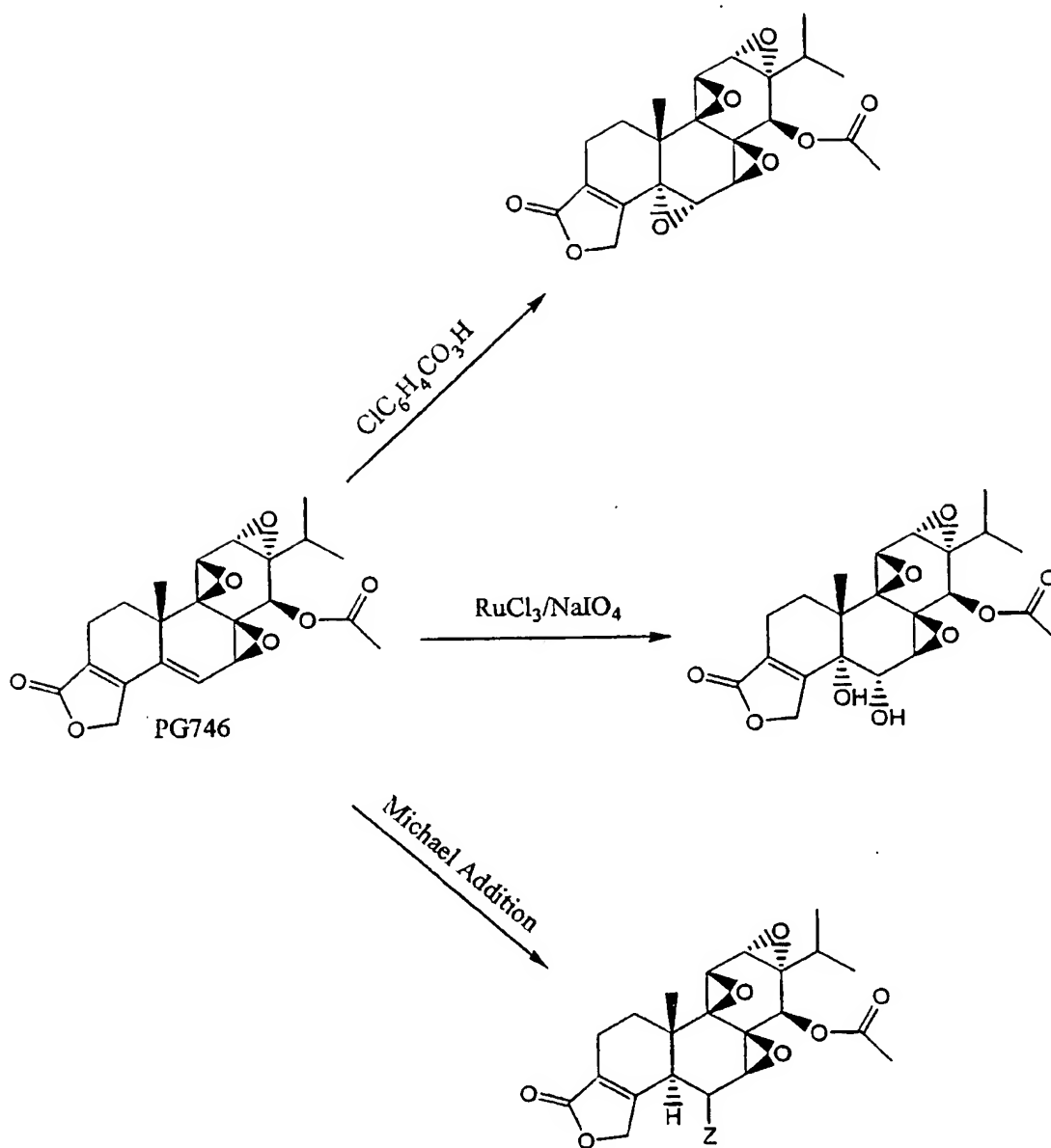
epoxide can be then be used to prepare compounds of formula I in which Y¹ is hydroxyl and Y² is selected from the group consisting of hydroxyl, halogen, cyano, N(R⁴)₂, and SR⁴, as defined above. Alternatively, compounds of formula I in which both Y¹ and Y² are hydroxyl can be prepared by direct hydroxylation of the 5,6-olefin of formula II, *e.g.* oxidation with periodate, as illustrated in Example 6 below.

Examples of the synthetic transformations described above are depicted in Schemes 1 and 2 below. Of course, these synthetic conversions are not limited to the use of the reagents shown, and various other reagents may be used to effect these conversions, as known and available to those skilled in the art. In addition, any free hydroxyl groups on the starting triptolide compounds may be protected as desired.

The relative stereochemistry of substituents at the 5 and/or 6 positions in the compounds of formula I will be determined by steric constraints, and are shown in Schemes 1 and 2 for the reactions depicted therein.



Scheme 1



III. Therapeutic Compositions

Formulations containing the compounds of the invention may take the form of solid, semi-solid, lyophilized powder, or liquid dosage forms, such as tablets, capsules, powders, sustained-release formulations, solutions, suspensions, emulsions, ointments, lotions, or aerosols, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions typically include a conventional pharmaceutical carrier or excipient and may additionally include other medicinal agents, carriers, or adjuvants.

Preferably, the composition will be about 0.5% to 75% by weight of a compound or compounds of the invention, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like. If desired, the composition may also contain minor amounts of non-toxic auxiliary substances such as wetting agents, emulsifying agents, or buffers.

The composition may be administered to a subject orally, transdermally or parenterally, *e.g.*, by intravenous, subcutaneous, intraperitoneal, or intramuscular injection. For use in oral liquid preparation, the composition may be prepared as a solution, suspension, emulsion, or syrup, being supplied either in liquid form or a dried form suitable for hydration in water or normal saline. For parenteral administration, an injectable composition for parenteral administration will typically contain the triptolide derivative in a suitable intravenous solution, such as sterile physiological salt solution.

Liquid compositions can be prepared by dissolving or dispersing the invention compounds (generally about 0.5% to about 20% by weight) and optional pharmaceutical adjuvants in a pharmaceutically acceptable carrier, such as, for example, aqueous saline, aqueous dextrose, glycerol, or ethanol, to form a solution or suspension.

The compounds may also be administered by inhalation, in the form of aerosol particles, either solid or liquid, preferably of respirable size. Such particles are sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about 1 to 10 microns in size, and preferably less than about 5 microns in size, are respirable. Liquid compositions for inhalation comprise the active agent dispersed in an aqueous carrier, such as sterile pyrogen free saline solution or sterile pyrogen free water. If desired, the composition may

be mixed with a propellant to assist in spraying the composition and forming an aerosol.

Methods for preparing such dosage forms are known or will be apparent to those skilled in the art; for example, see REMINGTON'S PHARMACEUTICAL SCIENCES (19th Ed., Williams & Wilkins, 1995). The composition to be administered will contain a quantity of
5 the selected compound in an effective amount for effecting immunosuppression in a subject or apoptosis in a targeted cell.

IV. Immunomodulating and Antiinflammatory Treatment

The compounds of the present invention are shown to have immunosuppressive
10 activity. As shown in Fig. 3, a compound of formula I, 5 α -hydroxy triptolide (designated PG701) inhibited IL-2 production in Jurkat cells (see Example 10) in a dose-dependent manner, at concentrations of about 10 nM or greater. A compound of formula II, 14-acetyl-5,6-didehydro triptolide (designated PG746) acts as a prodrug under
15 physiological conditions. In assay medium, the compound showed no significant activity relative to the medium control. However, after incubation for 16 hours with human serum, the compound inhibited IL-2 production in Jurkat cells in a dose-dependent manner, at concentrations of about 10 nM or greater.

The invention thus includes the use of the invention compounds as immunosuppressive agents, *e.g.* as an adjunct to transplant procedures or in treatment of
20 autoimmune disease.

Immunoregulatory abnormalities have been shown to exist in a wide variety of autoimmune and chronic inflammatory diseases, including systemic lupus erythematosus, chronic rheumatoid arthritis, type I and II diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease,
25 ulcerative colitis, bullous pemphigoid, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy and asthma. Although the underlying pathogenesis of each of these conditions may be quite different, they have in common the appearance of a variety of autoantibodies and self-reactive lymphocytes. Such self-reactivity may be due, in part, to a loss of the homeostatic controls under which the normal immune system operates.

30 Similarly, following a bone-marrow transplant or other transplant of hematopoietic stem cells from a donor tissue source containing mature lymphocytes, the transferred lymphocytes recognize the host tissue antigens as foreign. These cells become activated

and mount an attack upon the host (a graft-versus-host response) that can be life-threatening. Moreover, following an organ transplant, the host lymphocytes recognize the foreign tissue antigens of the organ graft and mount cellular and antibody-mediated immune responses (a host-versus-graft response) that lead to graft damage and rejection.

One result of an autoimmune or a rejection reaction is tissue destruction caused by inflammatory cells and the mediators they release. Anti-inflammatory agents such as NSAID's act principally by blocking the effect or secretion of these mediators but do nothing to modify the immunologic basis of the disease. On the other hand, cytotoxic agents, such as cyclophosphamide, act in such a nonspecific fashion that both the normal and autoimmune responses are shut off. Indeed, patients treated with such nonspecific immunosuppressive agents are as likely to succumb from infection as they are from their autoimmune disease.

The compositions of the present invention are useful in applications for which triptolide and its prodrugs and other derivatives have proven effective, e.g. in immunosuppression therapy, as in treating an autoimmune disease, preventing transplantation rejection, or treating or preventing graft-versus-host disease (GVHD). See, for example, co-owned U.S. Patent No. 6,150,539, which is incorporated herein by reference. Triptolide and the present derivatives are also useful for treatment of other inflammatory conditions, such as traumatic inflammation, and in reducing male fertility.

The compositions are useful for inhibiting rejection of a solid organ transplant, tissue graft, or cellular transplant from an incompatible human donor, thus prolonging survival and function of the transplant, and survival of the recipient. This use would include, but not be limited to, solid organ transplants (such as heart, kidney and liver), tissue grafts (such as skin, intestine, pancreas, gonad, bone, and cartilage), and cellular transplants (such as cells from pancreas, brain and nervous tissue, muscle, skin, bone, cartilage and liver).

The compositions are also useful for inhibiting xenograft (interspecies) rejection; *i.e.* in preventing the rejection of a solid organ transplant, tissue graft, or cellular transplant from a non-human animal, whether natural in constitution or bioengineered (genetically manipulated) to express human genes, RNA, proteins, peptides or other non-native, xenogeneic molecules, or bioengineered to lack expression of the animal's natural genes,

RNA, proteins, peptides or other normally expressed molecules. The invention also includes the use of a composition as described above to prolong the survival of such a solid organ transplant, tissue graft, or cellular transplant from a non-human animal.

- Also included are methods of treatment of autoimmune diseases or diseases having
- 5 autoimmune manifestations, such as Addison's disease, autoimmune hemolytic anemia, autoimmune thyroiditis, Crohn's disease, diabetes (Type I), Graves' disease, Guillain-Barre syndrome, systemic lupus erythematosus (SLE), lupus nephritis, multiple sclerosis, myasthenia gravis, psoriasis, primary biliary cirrhosis, rheumatoid arthritis and uveitis, asthma, atherosclerosis, Hashimoto's thyroiditis, allergic encephalomyelitis,
- 10 glomerulonephritis, and various allergies.

- Further uses may include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically mediated illnesses, such as psoriasis, atopic dermatitis, pemphigus, urticaria, cutaneous eosinophilias, acne, and alopecia areata; various eye diseases such as conjunctivitis,
- 15 uveitis, keratitis, and sarcoidosis; inflammation of mucous and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis, ischemic bowel diseases, inflammatory bowel diseases, and necrotizing enterocolitis; intestinal inflammations/allergies such as Coeliac diseases and ulcerative colitis; renal diseases such as interstitial nephritis, Good-pasture's syndrome, hemolytic-uremic syndrome and
- 20 diabetic nephropathy; hematopoietic diseases such as idiopathic thrombocytopenia purpura and autoimmune hemolytic anemia; skin diseases such as dermatomyositis and cutaneous T cell lymphoma; circulatory diseases such as arteriosclerosis and atherosclerosis; renal diseases such as ischemic acute renal insufficiency and chronic renal insufficiency, and Behcet's disease.

- 25 The compositions and method of the invention are also useful for the treatment of inflammatory conditions such as asthma, both intrinsic and extrinsic manifestations, for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma, particularly chronic or inveterate asthma (for example, late asthma and airway hyperresponsiveness). The composition and method may also be used for treatment of
- 30 other inflammatory conditions, including traumatic inflammation, inflammation in Lyme disease, chronic bronchitis (chronic infective lung disease), chronic sinusitis, sepsis associated acute respiratory distress syndrome, and pulmonary sarcoidosis. For treatment

of respiratory conditions such as asthma, the composition is preferably administered via inhalation, but any conventional route of administration may be useful.

In treating an autoimmune condition, the patient is given the composition on a periodic basis, *e.g.*, 1-2 times per week, at a dosage level sufficient to reduce symptoms and improve patient comfort. For treating rheumatoid arthritis, in particular, the composition may be administered by intravenous injection or by direct injection into the affected joint. The patient may be treated at repeated intervals of at least 24 hours, over a several week period following the onset of symptoms of the disease in the patient. The dose that is administered is preferably in the range of 1-25 mg/kg patient body weight per day, with lower amounts being preferred for parenteral administration, and higher amounts being preferred for oral administration. Optimum dosages can be determined by routine experimentation according to methods known in the art.

For therapy in transplantation rejection, the method is intended particularly for the treatment of rejection of heart, kidney, liver, cellular, and bone marrow transplants, and may also be used in the treatment of GVHD. The treatment is typically initiated perioperatively, either soon before or soon after the surgical transplantation procedure, and is continued on a daily dosing regimen, for a period of at least several weeks, for treatment of acute transplantation rejection. During the treatment period, the patient may be tested periodically for immunosuppression level, *e.g.*, by a mixed lymphocyte reaction involving allogenic lymphocytes, or by taking a biopsy of the transplanted tissue.

In addition, the composition may be administered chronically to prevent graft rejection, or in treating acute episodes of late graft rejection. As above, the dose administered is preferably 1-25 mg/kg patient body weight per day, with lower amounts being preferred for parenteral administration, and higher amounts for oral administration. The dose may be increased or decreased appropriately, depending on the response of the patient, and over the period of treatment, the ability of the patient to resist infection.

In treatment or prevention of graft-versus-host disease, resulting from transplantation into a recipient of matched or mismatched bone marrow, spleen cells, fetal tissue, cord blood, or mobilized or otherwise harvested stem cells, the dose is preferably in the range 0.25-2 mg/kg body weight/day, preferably 0.5-1 mg/kg/day, given orally or parenterally.

Also within the scope of the invention is a combination therapy comprising a compound of formula I or II and one or more conventional immunosuppressive agents.

These immunosuppressant agents within the scope of this invention include, but are not limited to, IMUREK™ (azathioprine sodium), brequinar sodium, SPANIDIN™ (gusperimus trihydrochloride, also known as deoxyspergualin), mizoribine (also known as bredinin), CELLCEPT™ (mycophenolate mofetil), NEORAL™ (Cyclosporin A; also marketed as a different formulation under the trademark SANDIMMUNE™), PROGRAF™ (tacrolimus, also known as FK-506), RAPIMMUNE™ (sirolimus, also known as rapamycin), leflunomide (also known as HWA-486), ZENAPAX™, glucocorticoids, such as prednisolone and its derivatives, antibodies such as orthoclone (OKT3), and antithymocyte globulins, such as thymoglobulins. The compounds are useful as potentiators when administered concurrently with another immunosuppressive drug for immunosuppressive treatments as discussed above. A conventional immunosuppressant drug, such as those above, may thus be administered in an amount substantially less (*e.g.* 20% to 50% of the standard dose) than when the compound is administered alone. Alternatively, the invention compound and immunosuppressive drug are administered in amounts such that the resultant immunosuppression is greater than what would be expected or obtained from the sum of the effects obtained with the drug and invention compound used alone. Typically, the immunosuppressive drug and potentiator are administered at regular intervals over a time period of at least 2 weeks.

The compositions of the invention may also be administered in combination with a conventional anti-inflammatory drug (or drugs), where the drug or amount of drug administered is, by itself, ineffective to induce the appropriate suppression or inhibition of inflammation.

Immunosuppressive activity of compounds *in vivo* can be evaluated by the use of established animal models known in the art. Such assays may be used to evaluate the relative effectiveness of immunosuppressive compounds and to estimate appropriate dosages for immunosuppressive treatment. These assays include, for example, a well-characterized rat model system for allografts, described by Ono and Lindsey (1969), in which a transplanted heart is attached to the abdominal great vessels of an allogeneic recipient animal, and the viability of the transplanted heart is gauged by the heart's ability to beat in the recipient animal. A xenograft model, in which the recipient animals are of a different species, is described by Wang (1991) and Murase (1993). A model for evaluating effectiveness against GVHD involves injection of normal F₁ mice with parental

spleen cells; the mice develop a GVHD syndrome characterized by splenomegaly and immunosuppression (Korngold, 1978; Gleichmann, 1984). Single cell suspensions are prepared from individual spleens, and microwell cultures are established in the presence and absence of concanavalin A to assess the extent of mitogenic responsiveness.

5

V. Anticancer Treatment

The compounds of the present invention are shown to have cytotoxic activity. As shown in Fig. 1, a compound of formula I, 5 α -hydroxy triptolide (designated PG701) induced apoptosis in Jurkat cells (see Example 8) in a dose-dependent manner. A

10 compound of formula II, 14-acetyl-5,6-didehydro triptolide (designated PG746) acts as a prodrug under physiological conditions. In assay medium (see Example 9), the compound showed no significant activity relative to the medium control. However, after incubation for 16 hours with human serum, the compound was clearly cytotoxic to Jurkat cells in this assay at concentrations of about 1 μ M or greater.

15 The invention thus includes the use of the invention compounds as cytotoxic agents, particularly to treat cancers. As used herein, "cancer" refers to all types of cancer or neoplasm or malignant tumors found in mammals especially humans, including leukemias, sarcomas, carcinomas and melanoma.

The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and
20 development of leukocytes and their precursors in the blood and bone marrow. The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. The term "melanoma" is taken to mean a tumor arising from
25 the melanocytic system of the skin and other organs. The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases.

Included, for example, are cancers involving cells derived from reproductive tissue (such as Sertoli cells, germ cells, developing or more mature spermatogonia, spermatids
30 or spermatocytes and nurse cells, germ cells and other cells of the ovary), the lymphoid or immune systems (such as Hodgkin's disease and non-Hodgkin's lymphomas), the hematopoietic system, and epithelium (such as skin, including malignant melanoma, and

gastrointestinal tract), solid organs, the nervous system, *e.g.* glioma (see Y.X. Zhou *et al.*, 2002), and musculo-skeletal tissue. The compounds may be used for treatment of various cancer cell types, including, but not limited to, brain, including medulloblastoma, head and neck, breast, colon, small cell lung, large cell lung, thyroid, testicle, bladder, prostate, 5 liver, kidney, pancreatic, esophageal, stomach, ovarian, cervical or lymphoma tumors. Treatment of breast, colon, lung, and prostate tumors is particularly contemplated.

The compositions may be administered to a patient afflicted with cancer and/or leukemia by any conventional route of administration, as discussed above. The method is useful to slow the growth of tumors, prevent tumor growth, induce partial regression of 10 tumors, and induce complete regression of tumors, to the point of complete disappearance. The method is also useful in preventing the outgrowth of metastases derived from solid tumors.

The compositions of the invention may be administered as sole therapy or with other supportive or therapeutic treatments not designed to have anti-cancer effects in the 15 subject. The method also includes administering the invention compositions in combination with one or more conventional anti-cancer drugs or biologic protein agents, where the amount of drug(s) or agent(s) is, by itself, ineffective to induce the appropriate suppression of cancer growth, in an amount effective to have the desired anti-cancer effects in the subject. Such anti-cancer drugs include actinomycin D, camptothecin, 20 carboplatin, cisplatin, cyclophosphamide, cytosine arabinoside, daunorubicin, doxorubicin, etoposide, fludarabine, 5-fluorouracil, hydroxyurea, gemcitabine, irinotecan, methotrexate, mitomycin C, mitoxantrone, paclitaxel, taxotere, teniposide, topotecan, vinblastine, vincristine, vindesine, and vinorelbine. Anti-cancer biologic protein agents include tumor necrosis factor (TNF), TNF-related apoptosis inducing ligand (TRAIL), 25 other TNF-related or TRAIL-related ligands and factors, interferon, interleukin-2, other interleukins, other cytokines, chemokines, and factors, antibodies to tumor-related molecules or receptors (such as anti-HER2 antibody), and agents that react with or bind to these agents (such as members of the TNF super family of receptors, other receptors, receptor antagonists, and antibodies with specificity for these agents).

30 Antitumor activity *in vivo* of a particular composition can be evaluated by the use of established animal models, as described, for example, in Fidler *et al.*, U.S. Patent No. 6,620,843. Clinical doses and regimens are determined in accordance with methods

known to clinicians, based on factors such as severity of disease and overall condition of the patient.

VI. Other Indications

5 The compounds of the present invention may also be used in the treatment of certain CNS diseases. Glutamate fulfills numerous physiological functions, including an important role in the pathophysiology of various neurological and psychiatric diseases. Glutamate excitotoxicity and neurotoxicity have been implicated in hypoxia, ischemia and trauma, as well as in chronic neurodegenerative or neurometabolic diseases, Alzheimer's
10 dementia, Huntington's disease and Parkinson's disease. In view of the reported neuroprotective effects of triptolide, particularly protection from glutamate-induced cell death (Q. He *et al.*, 2003; X. Wang *et al.*, 2003), compounds of the invention are envisioned to antagonize the neurotoxic action of glutamates and thus may be a novel therapy for such diseases.

15 Recent evidence from MS patients in relapse suggests an altered glutamate homeostasis in the brain. Neurotoxic events occurring in MS patients can be responsible for oligodendrocyte and neuronal cell death. Antagonizing glutamate receptor-mediated excitotoxicity by treatment with compounds of this invention may have therapeutic implications in MS patients. Other CNS diseases such as Guillain-Barre syndrome,
20 Meniere's disease, polyneuritis, multiple neuritis, mononeuritis and radiculopathy may also be treated with the compounds of the present invention.

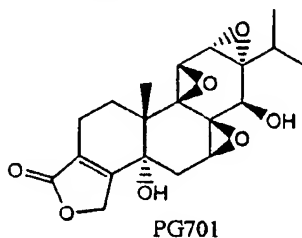
 The compounds of the present invention may also be used in the treatment of certain lung diseases. Idiopathic pulmonary fibrosis (PF) is a progressive interstitial lung disease with no known etiology. PF is characterized by excessive deposition of intracellular
25 matrix and collagen in the lung interstitium and gradual replacement of the alveoli by scar tissue as a result of inflammation and fibrosis. As the disease progresses, the increase in scar tissue interferes with the ability to transfer oxygen from the lungs to the bloodstream. A 14-succinimide ester of triptolide has been reported to block bleomycin-induced PF (G. Krishna *et al.*, 2001). Accordingly, the compounds of the present invention may be useful
30 for treatment of PF. Treatment of other respiratory diseases, such as sarcoidosis, fibroid lung, and idiopathic interstitial pneumonia is also considered. Other diseases involving the lung and envisioned to be treatable by compounds of this invention include Severe

Acute Respiratory Syndrome (SARS) and acute respiratory distress syndrome (ARDS). In particular, with respect to SARS, the reduction of virus content (SARS-CoV) before the peak of the disease process and the usefulness of corticosteroid treatment, as noted below, suggest that the development of the most severe, life-threatening effects of SARS may result from the exaggerated response of the body to the infection (immune hyperactivity) rather than effects of the virus itself. (See also copending and co-owned US provisional application S.N. 60/483,335, which is incorporated herein by reference.) Corticosteroid treatment has been used in SARS patients to suppress the massive release of cytokines that may characterize the immune hyperactive phase, in the hope that it will stop the progression of pulmonary disease in the next phase. Corticosteroid treatment has produced good clinical results in reduction of some of the major symptoms of SARS. However, there are several treatment-related side effects, and there is a clear need for more selective immunosuppressive and/or antiinflammatory agents.

15 EXAMPLES

The following examples illustrate but are not intended in any way to limit the invention.

Example 1: Synthesis of 5- α -hydroxytriptolide (PG701)



20

To a solution of triptolide (437.6mg, 1.21mmol) in 1,4-dioxane (35mL) was added selenium dioxide (305.1mg, 2.75mmol). The reaction mixture was stirred at 90°C under N₂ for 70 hrs. After cooling to room temperature, the reaction mixture was filtered through Celite and concentrated under vacuum. The crude product was purified via preparative TLC (ethyl acetate/dichloromethane 3:7) to yield the desired product (211.7mg, 46.3%).

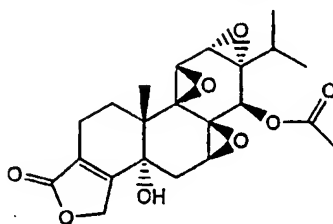
Analytical TLC: R_f = 0.30 (ethyl acetate/hexanes/methanol 1:1:0.1).

IR (KBr): 3469.5, 2962.6, 2886.5, 1763.3, 1736.3, 1676.9, 1459.5, 1432.4, 1383.1, 1365.1, 1349.3, 1301.6, 1277.0, 1269.4, 1246.5, 1203.2, 1160.4, 1112.9, 1079.7, 1030.9, 988.0, 951.1, 945.3, 943.9, 907.7, 877.2, 855.2, 841.2, 812.7, 797.8, 780.4, 748.2, 656.1, 645.0, 605.5, 580.4, 555.7, 524.3, 498.7, 483.9, 469.5, 439.4, 428.9, 414.9, 407.1
5 cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.90 (d, 3H, 16-CH₃), 1.02 (d, 3H, 17-CH₃), 1.18 (s, 3H, 20-CH₃), 1.28 (m, 1H, 1-CHb), 1.77 (m, 1H, 1-CHa), 2.08-2.42 (m, 5H, 2-CH₂, 6-CH₂ and 15-CH), 2.81 (t, 2H, 5-OH and 14-OH), 3.37 (d, 1H, 7-CH), 3.48 (d, 1H, 14-CH), 3.63 (d, 1H, 12-CH), 3.96 (d, 1H, 11-CH), 4.82 (m, 2H, 19-CH₂) ppm.

10

Example 2: Synthesis of 14-acetyl-5-α-hydroxytriptolide

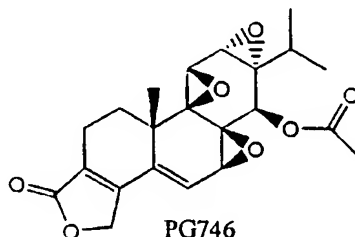


To a solution of 5-α-hydroxytriptolide (PG701, 98.3mg, 0.261mmol), 4-dimethylaminopyridine (DMAP, 45.2mg) and triethylamine (TEA, 0.50mL) in
15 dichloromethane (5.0mL) was added acetic anhydride (0.247mL, 2.61mmol, 10.0eq) at room temperature under nitrogen. After stirring for 4-5 hrs at room temperature, methanol (1.0mL) was added, and the reaction mixture was concentrated under vacuum. The crude product was purified using preparative TLC (ethyl acetate/hexanes/methanol 1.0:1.0:0.1) to yield the desired product (95.7mg, 87.6%).

20 Analytical TLC: R_f = 0.43 (ethyl acetate/hexanes/methanol 1:1:0.1).

IR (KBr): 3491.2, 2970.1, 1752.5, 1685.8, 1676.8, 1474.3, 1459.2, 1439.1, 1375.0, 1230.4, 1154.7, 1033.3, 994.0, 951.2, 923.6, 899.9, 870.1, 777.3, 750.0, 646.0, 618.7, 605.4, 570.3, 547.3, 529.2, 506.8, 499.0, 491.9, 476.5, 463.3, 447.4, 431.4, 418.5, 401.2
cm⁻¹.

25 ¹H NMR (300 MHz, CDCl₃): δ = 0.86 (d, 3H, 16-CH₃), 0.97 (d, 3H, 17-CH₃), 1.12 (s, 3H, 20-CH₃), 1.30 (dd, 1H, 1-CHb), 1.74 (m, 1H, 1-CHa), 1.90 (m, 1H, 15-CH), 2.06-2.40 (m, 4H, 2-CH₂ and 6-CH₂), 2.80 (s, 1H, 5-OH), 3.46 (d, 1H, 7-CH), 3.62 (d, 1H, 12-CH), 3.86 (d, 1H, 11-CH), 4.82 (m, 2H, 19-CH₂), 5.14 (d, 1H, 14-CH) ppm.

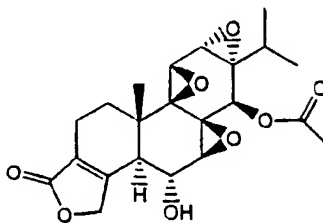
Example 3: Synthesis of 14-acetyl-5,6-didehydrotriptolide (PG746)

5 To a solution of 14-acetyl-5- α -hydroxytriptolide, prepared as described in Example 2 (10.5mg, 0.025mmol), in dichloromethane (0.50mL) at 0°C was added (diethylamino)sulfur trifluoride (DAST, 4.3 μ L, 0.033mmol, 1.3eq). The reaction mixture was stirred at 0°C under N₂ for 40 mins. Saturated NaHCO₃ aqueous solution (0.2mL) diluted with 0.3 mL H₂O was added to the reaction mixture at 0°C. The mixture was then
10 extracted with dichloromethane (1.5, 2x2.0mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under vacuum. The crude product was purified using preparative TLC (ethyl acetate/hexanes/methanol 40:60:5.0) to yield the desired product (4.0mg, 39.8%).

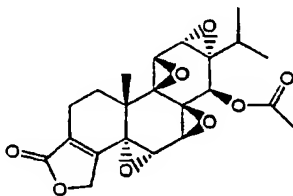
Analytical TLC: R_f = 0.71 (ethyl acetate/hexanes/methanol 1:1:0.1).

15 IR (KBr): 2969.7, 2880.3, 1747.8, 1686.2, 1655.3, 1649.3, 1625.6, 1438.1, 1371.1, 1305.7, 1224.9, 1154.4, 1101.0, 1081.7, 1028.1, 995.0, 975.5, 952.5, 938.3, 921.1, 895.4, 870.5, 846.2, 821.1, 785.5, 747.1, 638.4, 614.4, 588.4, 560.4, 546.2, 523.2, 511.1, 498.7, 480.5, 430.1, 416.5 cm⁻¹.

H¹ NMR (300 MHz, CDCl₃): δ = 0.86 (d, 3H, 16-CH₃), 0.98 (d, 3H, 17-CH₃), 1.30 (s, 3H, 20-CH₃), 1.40 (m, 1H, 1-CHb), 1.49 (m, 1H, 1-CHa), 1.91 (m, 1H, 15-CH), 2.33 (m, 1H, 2-CHb), 2.46 (m, 1H, 2-CHa), 3.54 (m, 2H, 12-CH and 7-CH), 3.80 (d, 1H, 11-CH), 4.90 (m, 2H, 19-CH₂), 5.20 (s, 1H, 14-CH), 5.99 (d, 1H, 6-CH) ppm.
20

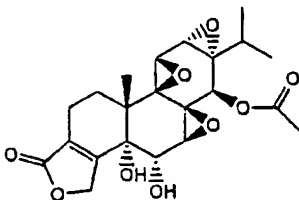
Example 4: Synthesis of 14-acetyl-6- α -hydroxytriptolide

To a solution of 14-acetyl-5,6-didehydrotriptolide (PG746) (prepared as described in
5 Example 3) in THF at room temperature under N₂ was added BH₃·THF (1.0M in THF).
The reaction mixture was stirred at room temperature for 3 hrs, and NaOH (1.0N in H₂O)
was then added, followed by the addition of H₂O₂ (50% by weight in H₂O). The mixture
was extracted with dichloromethane for three times, and the combined organic layers
were dried over anhydrous Na₂SO₄. After removal of solvent, the crude product was
10 purified with preparative TLC.

Example 5: Synthesis of 14-acetyl-5,6-epoxytriptolide

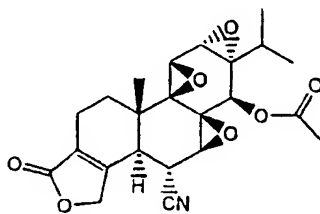
15 To a solution of 14-acetyl-5,6-didehydrotriptolide (PG746) in dichloromethane at
room temperature is added *m*-chloroperbenzoic acid (MCPBA, 1.25eq). The reaction
mixture is stirred overnight at room temperature, filtered, washed with 10% NaHCO₃
solution, and dried over anhydrous Na₂SO₄. After removal of solvent, the crude product
is purified using preparative TLC.

20

Example 6: Synthesis of 14-acetyl-5 α ,6 α -dihydroxytriptolide

To a solution of 14-acetyl-5,6-didehydrotriptolide (PG746) in ethyl acetate/acetonitrile (1:1) at room temperature is added a solution of $\text{RuCl}_3 \cdot x\text{H}_2\text{O}$ and NaIO_4 in H_2O . A brown precipitate forms. The reaction mixture is stirred at room temperature for 15 mins and then poured into saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$. The mixture is
5 extracted with dichloromethane three times, and the combined organic layers are dried over anhydrous Na_2SO_4 . After removal of solvent, the crude product is purified using preparative TLC.

Example 7: Synthesis of 14-acetyl-6-cyanotriptolide



10

To a solution of 14-acetyl-5,6-didehydrotriptolide (PG746) in toluene at room temperature is added Et_2AlCN (1.0M in toluene, 2.0eq). The reaction mixture is stirred overnight at 45°C , cooled to room temperature, diluted with dichloromethane, washed
15 with 10% NaHCO_3 solution, and dried over anhydrous Na_2SO_4 . After removal of solvent, the crude product is purified using preparative TLC.

Example 8: Annexin V Apoptosis Assay

Test samples were diluted to 1 mM in complete tissue culture medium (RPMI 1640
20 medium plus 5 % heat-inactivated fetal calf serum, 1% HEPES, 1% pen/strep, 1% glutamine). Aliquots were placed in microculture plates and serial dilutions were prepared so that the final concentration would encompass the range of 2 to 6,000 nM with half-log increments. Cells from an exponentially expanding culture of the Jurkat human T lymphoma cell line (#TIB-152 obtained from American Type Culture Collection,
25 Manassas, VA) were harvested, washed once by centrifugation, re-suspended in complete tissue culture medium, and further diluted to a concentration of 1×10^6 cells/ml. A volume of 100 μl of the cells (1×10^5 cells) was added to wells containing 100 μl of the diluted compounds, and the plates were incubated at 37°C in a 5% CO_2 incubator.

After 24 hours, the plates were centrifuged to pellet the cells, and the cells were washed twice with 2% heat-inactivated fetal calf serum in PBS. To each well, 500ul of binding buffer was added, according to the Annexin V assay procedure (BioVision, Inc., Mountain View, CA). Next, 5 μ l of the fluorescein isothiocyanate (FITC) conjugate of Annexin V (BioVision, Inc.) was added to each well, followed by 5 minutes of incubation in the dark. In some assays, propidium iodide (BioVision, Inc.) was added at this stage to check for necrotic cells. The contents of the wells were individually transferred into test tubes, and apoptosis was analyzed using a FACSCalibur flow cytometer (BD Immunocytometry Systems, San Jose, CA). Cells positive for Annexin V binding were considered to be apoptotic, and the data were calculated as percent apoptotic cells.

Data were plotted as the concentration of compound versus percent apoptotic cells. Data for invention compound 5 α -hydroxy triptolide (designated PG701) and triptolide (designated PG490) are given in Fig. 1.

15 Example 9. Cytotoxicity (MTT) Assay

Test compounds were dissolved in DMSO at a concentration of 20 mM. Further dilutions were done in RPMI1640 medium (GIBCO, Rockville, MD) supplemented with 10% Fetal Calf Serum (HyClone Laboratories, Logan, UT). Invention compound 14-acetyl-5,6-didehydrotriptolide (designated PG746) was tested with and without a previous 16-hr incubation in human serum. Such incubation is expected to substantially convert the 14-acetyl group of PG746 to a 14-hydroxy group.

Cytotoxicity of the compounds was determined in a standard MTT assay using Cell Proliferation Kit I (#1 465 007, Roche Diagnostics, Mannheim, Germany). Accordingly, human T cell lymphoma (Jurkat) cells (4×10^5 per well) were cultured for 24h, in 96-well tissue culture plates, in the presence of serial three-fold dilutions of test compounds or medium containing the same concentration of DMSO as in the test samples at each dilution point. The cultures were then supplemented with 10 μ l/well MTT reagent for 4h and then with 0.1 ml/well solubilizing reagent for an additional 16h. Optical density at 570 nm was measured on a ThermoScan microplate reader (Molecular Devices, Menlo Park, CA).

The data is presented as OD₅₇₀ values versus concentration of the compounds. The results for invention compound 14-acetyl-5,6-didehydrotriptolide (designated PG746) and

triptolide (designated PG490) are given in Fig. 2.

Example 10. IL-2 Inhibition Assay

Test compounds were diluted to 1 mM in complete tissue culture medium. Aliquots
5 were placed in microculture plates coated with anti-CD3 antibody (used to stimulate the production of IL-2 by Jurkat cells), and serial dilutions were prepared so that the final concentration would encompass the range of 0.001 to 10,000 nM in log increments.

Invention compound 14-acetyl-5,6-didehydrotriptolide (designated PG746) was tested with and without a previous 16-hr incubation in human serum. Such incubation is
10 expected to substantially convert the 14-acetyl group of PG746 to a 14-hydroxy group.

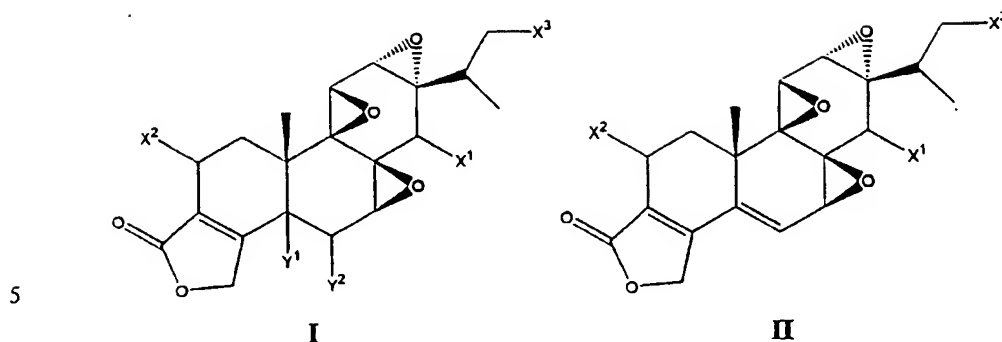
Cells from an exponentially expanding culture of Jurkat human T cell line (#TIB-152 obtained from American Type Culture Collection, Manassas, VA) were harvested, washed once by centrifugation, re-suspended in complete tissue culture medium, and diluted to a concentration of 10^7 cells/ml. A volume of 50 μ l of Jurkat cells was added to
15 wells containing 100 μ l of the diluted compounds, and the cells were stimulated with 50 ng/ml PMA and 10 mg/ml PHA. The plates were incubated at 37°C in a 5% CO₂ incubator. After 24 hours, the plates were centrifuged to pellet the cells, 150 μ l of supernatant was removed from each well, and the samples were stored at -20 °C. The stored supernatants were analyzed for human IL-2 concentration using the Luminex 100
20 (Luminex Corporation, Austin, TX), Luminex microspheres coupled with anti-IL-2 capture antibody, and fluorochrome-coupled anti-IL-2 detection antibody. The data were expressed as pg/ml of IL-2.

The data were plotted as the concentration of compound versus IL-2 concentration. The results for PG701 (5 α -hydroxy triptolide), compared with PG490 (triptolide) and a
25 solvent control, are given in Fig. 3. The results for PG746 (14-acetyl-5,6-didehydro triptolide), with and without incubation in human serum, compared with PG490 (triptolide) and a solvent control, are given in Fig. 4.

While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications may be made without
30 departing from the invention.

IT IS CLAIMED:

1. A compound having the structure I or II:



where

- X^1 is OR^1 , where R^1 is selected from hydrogen, $C(=O)R^2$, and $C(=O)OR^2$, where R^2 is selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl;
- 10

X^2 and X^3 are independently OR^1 or hydrogen, at least one of X^2 and X^3 being hydrogen; and where

- (i) Y^1 is hydrogen and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl, ethenyl, $-CH_2COOR^4$, $N(R^4)_2$, and SR^4 , where each R^4 is
- 15 independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, or, in the case of $N(R^4)_2$, taken together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, wherein the ring atoms include at most 3 heteroatoms; or
- 20 (ii) Y^1 is hydroxyl and Y^2 is selected from the group consisting of hydrogen, hydroxyl, halogen, cyano, $N(R^4)_2$, and SR^4 ; or
- (iii) Y^1 and Y^2 taken together form an epoxide ring.

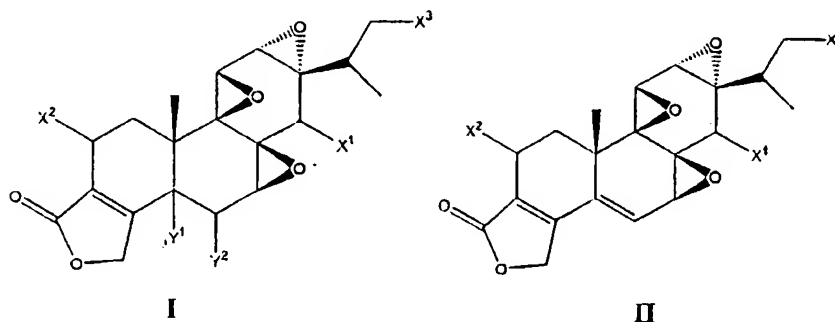
2. A compound as recited in claim 1, where R^1 is selected from hydrogen and $C(=O)R^2$.

3. A compound as recited in claim 2, where R^2 is selected from alkyl, aryl, aralkyl, and alkoxyalkyl.

4. A compound as recited in claim 3, where R^2 is selected from lower alkyl, phenyl, and benzyl.
- 5 5. A compound as recited in claim 2, where R^1 is hydrogen.
6. A compound as recited in claim 1, wherein each of X^2 and X^3 is hydrogen.
7. A compound as recited in claim 1, having the structure II.
- 10 8. A compound as recited in claim 7, wherein each of X^2 and X^3 is hydrogen.
9. A compound as recited in claim 1, having the structure I.
- 15 10. A compound as recited in claim 9, wherein each of X^2 and X^3 is hydrogen.
11. A compound as recited in claim 10, wherein Y^1 is hydrogen and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl, ethenyl, $-\text{CH}_2\text{COOR}^4$, $\text{N}(\text{R}^4)_2$, and SR^4 .
- 20 12. A compound as recited in claim 11, wherein Y^2 is hydroxyl, fluoro, chloro, bromo, cyano, $-\text{CH}_2\text{COOR}^4$, or $\text{N}(\text{R}^4)_2$.
13. A compound as recited in claim 12, wherein Y^2 is hydroxyl, fluoro, chloro, bromo, or
25 cyano.
14. A compound as recited in claim 13, wherein Y^2 is hydroxyl.
15. A compound as recited in claim 13, wherein Y^2 is cyano.
- 30 16. A compound as recited in claim 10, wherein Y^1 is hydroxyl and Y^2 is selected from the group consisting of hydrogen, hydroxyl, halogen, cyano, $\text{N}(\text{R}^4)_2$, and SR^4 .

17. A compound as recited in claim 16, wherein Y^2 is hydrogen, hydroxyl, fluoro, chloro, bromo, or cyano.
- 5 18. A compound as recited in claim 17, wherein Y^2 is hydrogen.
19. A compound as recited in claim 17, wherein Y^2 is hydroxyl.
20. A compound as recited in claim 10, wherein Y^1 and Y^2 taken together form an
10 epoxide ring.
21. A compound as recited in claim 1, wherein the groups defined as R^2 , R^3 , and R^4 , when selected from alkyl, alkenyl, and alkynyl, have up to six carbon atoms; when selected from cycloalkyl, have 3 to 7 carbon atoms; when selected from cycloalkenyl,
15 have 5 to 7 carbon atoms; and when selected from aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, have alkyl components having up to six carbon atoms.
22. A compound as recited in claim 21, wherein said alkyl, alkenyl, and alkynyl groups or components have up to four carbon atoms.
20
23. A compound as recited in claim 21, wherein said alkyl, alkenyl, and alkynyl groups or components have one or two carbon atoms.
24. A compound as recited in claim 1, wherein each of the groups defined as R^2 , R^3 , and
25 R^4 is independently selected from alkyl, aryl, aralkyl, and alkoxyalkyl.

25. A method of effecting immunosuppression, comprising administering to a subject in need of such treatment, an effective amount of a compound of formula I or II:



where

X^1 is OR^1 , where R^1 is selected from hydrogen, $C(=O)R^2$, and $C(=O)OR^2$, where R^2 is selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl;

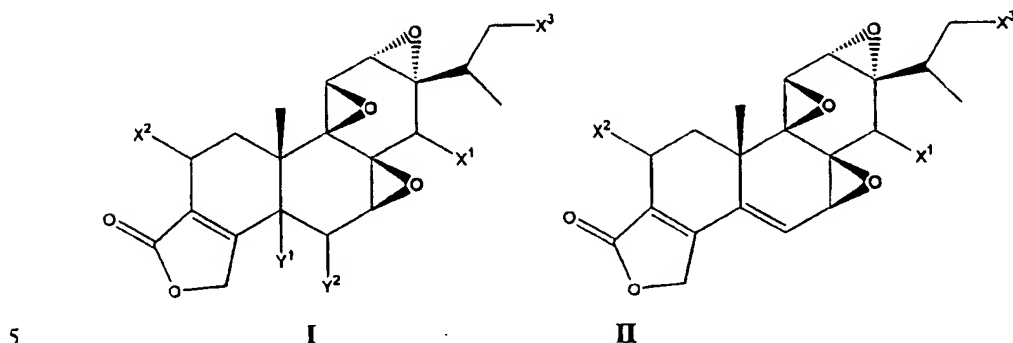
X^2 and X^3 are independently OR^1 or hydrogen, at least one of X^2 and X^3 being hydrogen; and where

(i) Y^1 is hydrogen and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl, ethenyl, $-CH_2COOR^4$, $N(R^4)_2$, and SR^4 , where each R^4 is independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, or, in the case of $N(R^4)_2$, taken together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, wherein the ring atoms include at most 3 heteroatoms; or

(ii) Y^1 is hydroxyl and Y^2 is selected from the group consisting of hydrogen, hydroxyl, halogen, cyano, $N(R^4)_2$, and SR^4 ; or

(iii) Y^1 and Y^2 taken together form an epoxide ring.

26. A method of inducing apoptosis in a cell, comprising contacting said cell with an effective amount of a compound of formula I or II:



where

X¹ is OR¹, where R¹ is selected from hydrogen, C(=O)R², and C(=O)OR², where R² is selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl;

10 X^2 and X^3 are independently OR¹ or hydrogen, at least one of X^2 and X^3 being hydrogen; and where

(i) Y^1 is hydrogen and Y^2 is selected from the group consisting of hydroxyl, halogen, cyano, nitromethyl, ethenyl, $-\text{CH}_2\text{COOR}^4$, $\text{N}(\text{R}^4)_2$, and SR^4 , where each R^4 is independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, aralkyl, hydroxyalkyl, alkoxyalkyl, aryloxyalkyl, and acyloxyalkyl, or, in the case of $\text{N}(\text{R}^4)_2$, taken together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, wherein the ring atoms include at most 3 heteroatoms; or

(ii) Y^1 is hydroxyl and Y^2 is selected from the group consisting of hydrogen, hydroxyl, halogen, cyano, $N(R^4)_2$, and SR^4 ; or

(iii) Y^1 and Y^2 taken together form an epoxide ring.

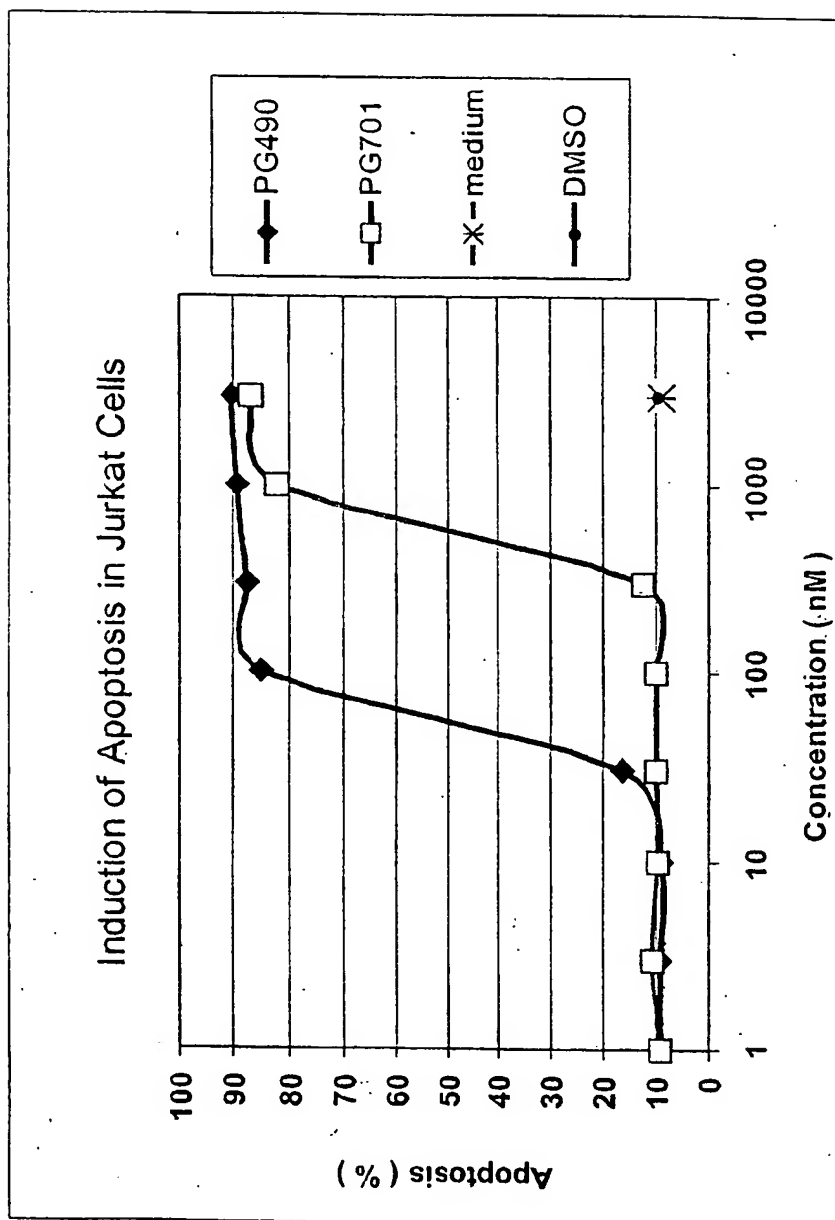


Fig. 1

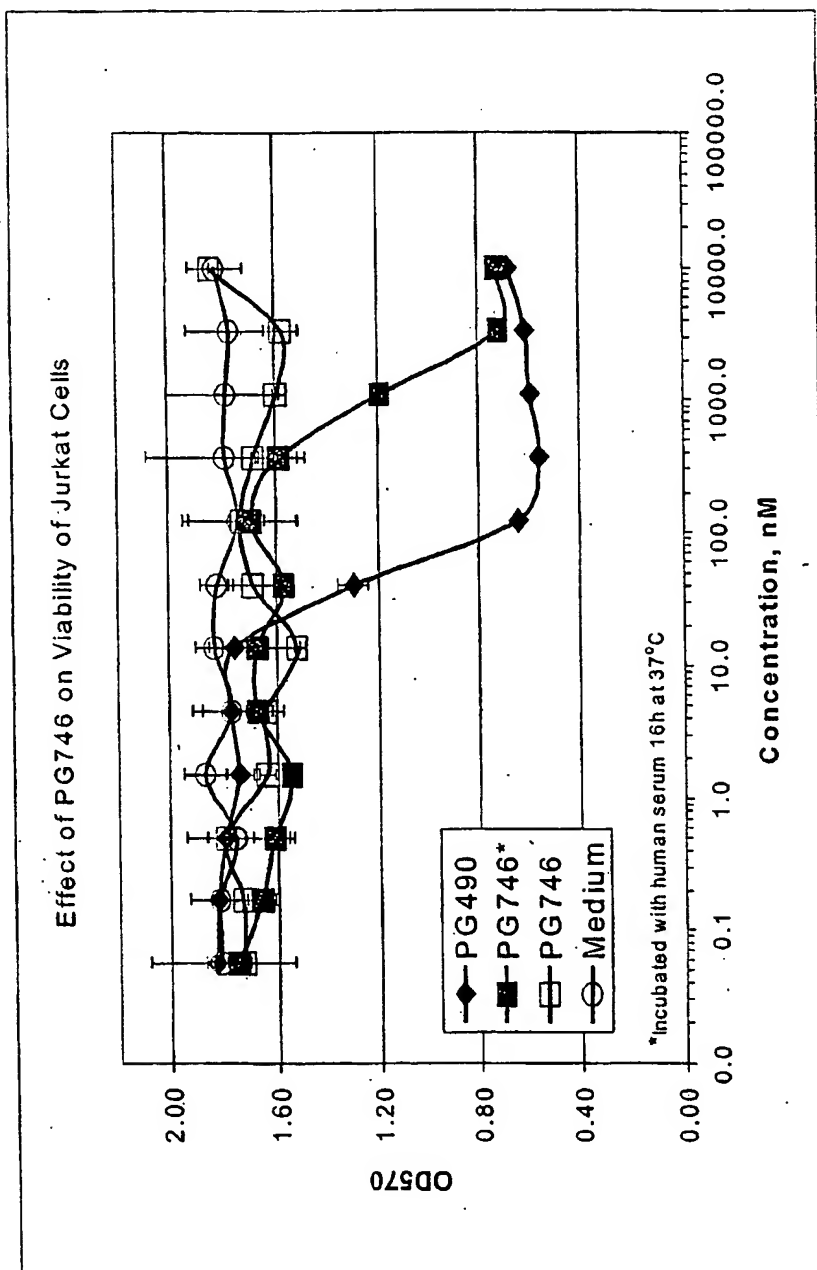


Fig. 2

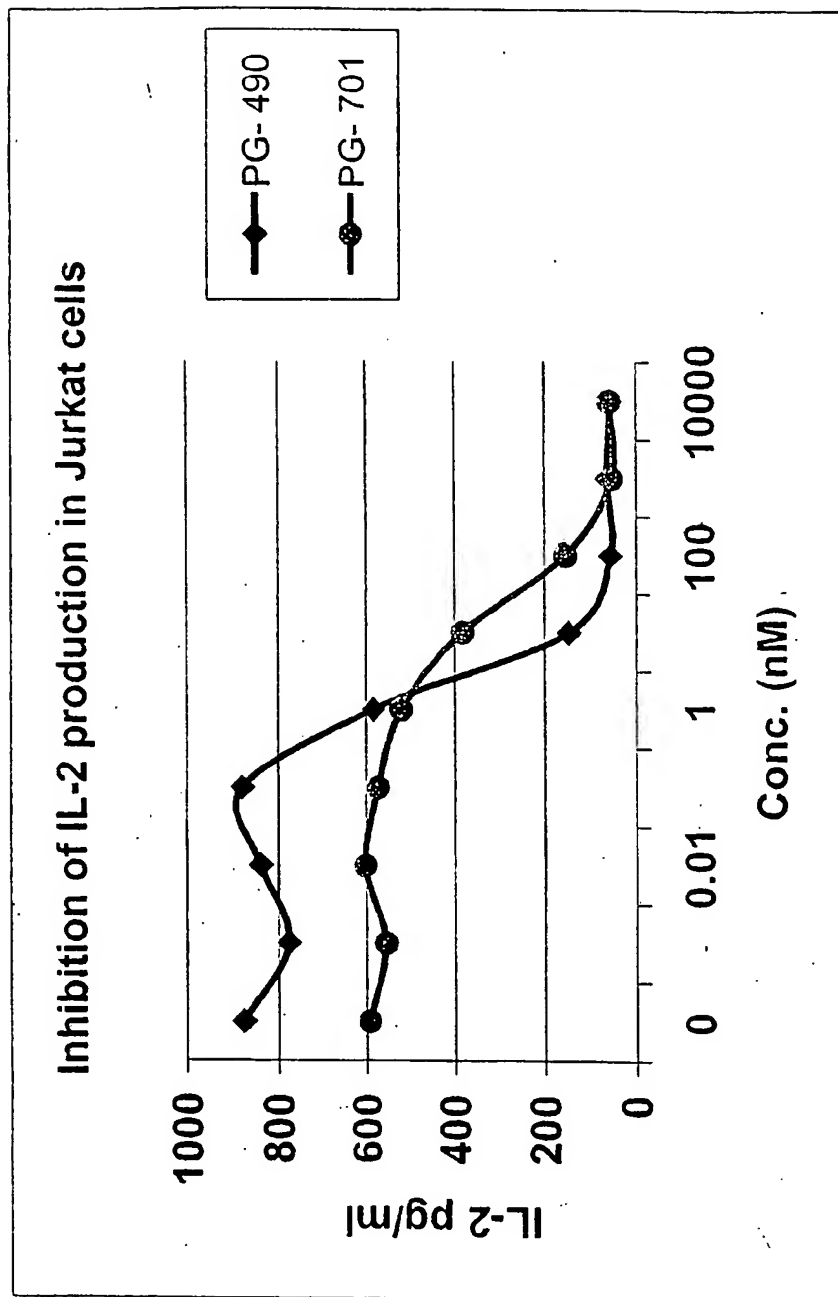


Fig. 3

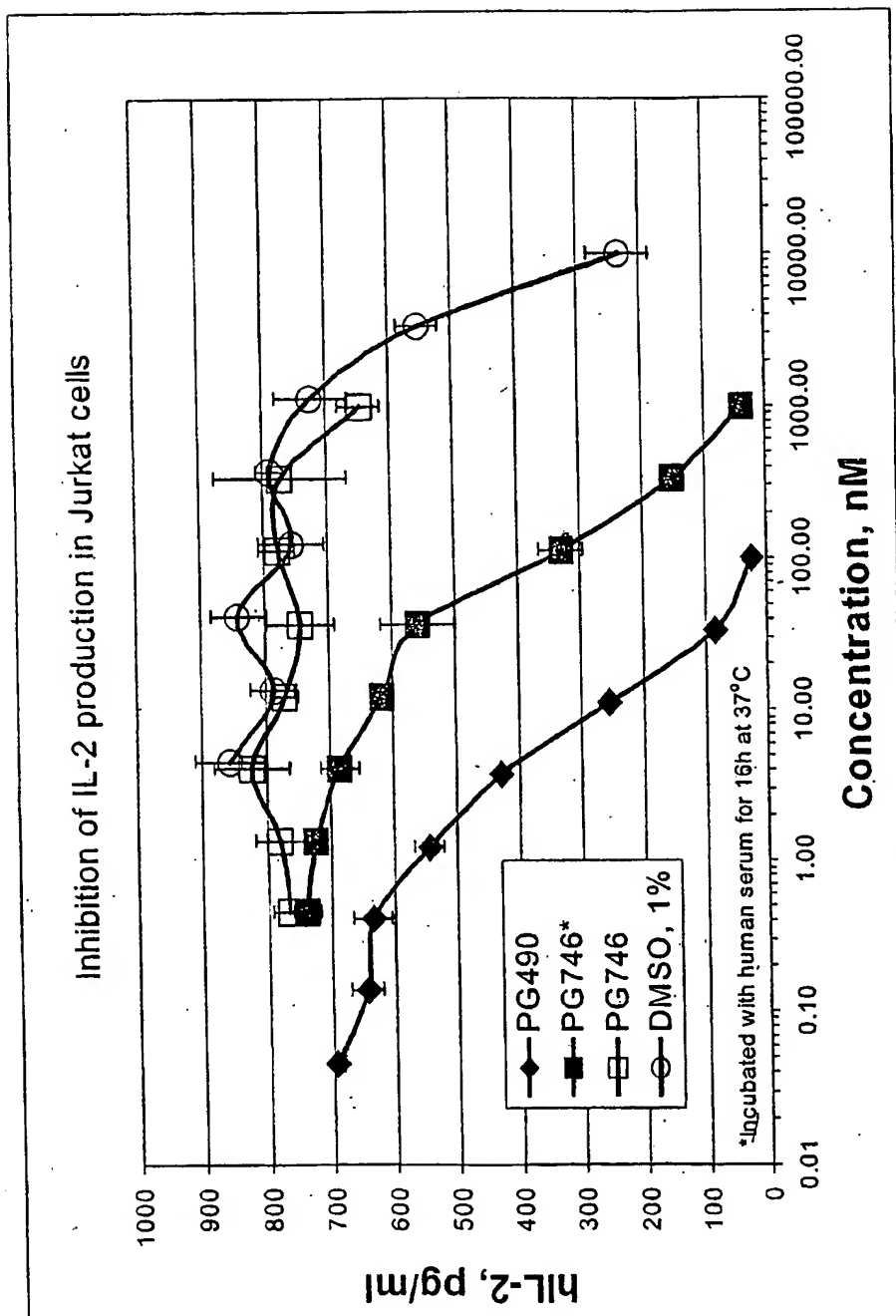


Fig. 4

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(57) Abstract: Compounds useful as immunosuppressive, anti-inflammatory and anticancer agents and methods of their preparation and use are described. The compounds are analogs or derivatives of triptolide and related compounds, modified at the 5- and/or 6-position relative to the naturally occurring compounds.

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CAS ONLINE-structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NING et al, Biotransformation of Triptolide by Cunninghamella Blakesleanna, Tetrahedron, June 2003, vol. 59, No. 23, pages 4209-4213, especially page 4209 and 4210.	1-6,9,10,16-18 and 21-25
P,A	WO 2004/058770 A1 (LI et al) 15 July 2004 (15.07.2004), entire document	----- 1-14 and 16-26

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